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MICROSCOPE SLIDE COVER WITH INTEGRATED RESERVOIR**Field of the invention**

The present invention relates to a cover for a substrate, and in one form a cover for use with a microscope slide.

5 Background of the invention

Microscope slides are commonly used to view samples of material under a microscope. The samples may contain human tissue, and may require treatment such as staining, so that properties of the sample can be identified. Other materials such as DNA, RNA, or proteins may be included on the slide.

- 10 It is common for several reactions to be undertaken on a sample on a slide. Once the reactions have taken place the slide may be viewed under a microscope. Performing the reactions on the slide can be difficult to automate, as the tissue samples require careful preparation and certain reactions require carefully controlled environments.

Summary of the Present Invention

- 15 In accordance with the present invention, there is provided a cover for a substrate including:

a body defining a cavity, for positioning over the substrate to form a reaction chamber; and

- 20 a projection extending from the body to define a fluid reservoir, when the cover is fitted to the substrate, the fluid reservoir being in fluid communication with the cavity.

Preferably the cavity extends the full width of a sample holding region on the substrate.

Preferably, a protrusion extends from the projection, to assist in wicking fluid into the reservoir.

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Preferably, the reservoir is defined between the projection, which is spaced from the substrate, and legs located at sides edge of the cover.

In one form the projection is formed from two sections, the first section is angled at least at substantially 60° relative to the cavity and the second section is angled at least
5 at substantially 15°.

In one form, the cover further includes a second reservoir, at an opposite end of the cover.

Preferably wall portions are located at the edge of the cover, surrounding the cavity on two or more sides.

10 In one form the legs extend along the sides of the cover to form the wall portions.

In a preferred form, the cover includes a locator for controlling and locating the cover, the locator being arranged at an end of the cover opposite the projection.

In one form the cavity extends to an end edge of the cover adjacent the locator.

In one form the cover is supported on the substrate by the wall portions.

15 Preferably, the cover is made from a polymer material.

In one form the cavity includes a coating of reduced surface roughness than the polymer material.

In another form the cavity includes a coating with reduced porosity.

In another form the cavity has one or more coatings.

20 Preferably a first coating is a material having similar properties to the material of the slide.

Preferably the first coating is silicon dioxide.

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Preferably a second coating is placed intermediate a first coating to provide improved contact properties between the cover and first coating.

Preferably, the cover has associated wing structures that allow the cover to be engaged and pivoted relative to the substrate so as to open the reaction chamber and
5 allow the slide to be cleared of fluid.

In another aspect, there is provided a combination of a substrate and a cover, as described above, wherein the cavity of the cover is arranged to face the substrate so as to form a reaction chamber.

In yet another aspect, there is provided a method of treatment of a sample on a sample
10 holding region of a substrate, including locating a cover, as described above, over the substrate, so that the cavity of the cover faces the substrate to form a reaction chamber over the sample holding region, and depositing fluid into the fluid reservoir to allow the fluid to be drawn into the reaction chamber, as required.

Preferably, the method further includes sliding the cover relative to the substrate to
15 vary a degree of overlap between the cover and the sample holding region, which results in a corresponding variation in the reaction chamber volume.

Preferably, the method further includes sliding the cover relative to the substrate until wing structures associated with the cover are engaged and lifted relative to the substrate to pivot the cover into an open condition, and allow fluid to drain from the
20 reaction chamber.

Brief Description of the Drawings

The invention is described, by way of non-limiting example only, with reference to the accompanying drawings, in which:

Figure 1 shows an example of a microscope slide;

25 Figures 2 (a)-(c) show top, side and bottom views of a first example of a cover for a slide;

Figure 3 shows a perspective view of the cover of Figure 2;

Figures 4 (a)-(c) show further views of the cover of Figure 2 located on the slide of Figure 1;

Figure 5 is a perspective view of the cover and slide arrangement of Figure 4,
5 showing a cutaway section of the cover;

Figure 6 shows a schematic cross section of the cover and slide of Figure 5;

Figure 7 shows a perspective view of a tray adapted to locate covers and slides;

Figures 8 (a) and (b) show schematic top and sectional side views, respectively, of a further example of a cover;

10 Figures 9 (a) and (b) show schematic top and sectional side views, respectively, of a further example of a cover;

Figures 10 (a) and (b) show schematic top and sectional side views, respectively, of a further example of a cover;

Figures 11 (a) and (b) show schematic top and sectional side views, respectively, of a
15 further example of a cover;

Figures 12 (a) and (b) show schematic top and sectional side views, respectively, of a further example of a cover;

Figures 13 (a) and (b) shows schematic top and sectional side views, respectively, of a further example of a cover;

20 Figures 14 (a) and (b) show top and bottom perspective views, respectively, of a further example of a cover;

Figure 15 shows a schematic side view of a nose portion of a cover;

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Figure 16 (a) and (b) show schematic top and sectional side views, respectively, of a further example of a cover;

Figure 17 shows a schematic side view of a further example of a nose portion of a cover;

5 Figure 18 shows the cover of Figure 2 mounted to the tray of figure 7;

Figures 19 (a)-(c) shows the cover of Figure 2 in various positions over the slide of Figure 1; and

Figure 20(a) and (b) show a bottom perspective view and enlarged partial perspective view, respectively of a modified cover.

10 Detailed Description

A microscope slide 1 is shown in Figure 1 as including an upper surface 2 containing a sample 3. The slide 1 is identified by a unique bar code 4. The sample 3, such as a thinly sliced tissue section, is located on the slide 1 in a sample holding region 5, which needs to be covered by a cover, such as shown in Figure 2, for subsequent
15 application of test fluids and the like.

Figures 2 (a)-(c) and Figure 3 show a cover 10 as having a body 12, a fluid receiving zone 14, a locating means 16 and a cavity 18 on an underside face 19. Surrounding the cavity 18 on two sides is a wall portion 20. At one end of the cover 10, the wall portion 20 joins with legs 21 which extend upwardly and away from the face 19. The
20 legs 21 are spanned by a projection 13 which defines a fluid reservoir 17, between an underside of the projection and the legs 21.

The cover 10 is shown fitted to a slide 1 in Figures 4 and 5. The fluid reservoir 17 is shown most clearly in Figure 4 (c) where a detailed view of part of a section A-A taken across the cover 10 and slide 1 is illustrated. The projection 13, with leg 21 at
25 either end, is raised relative to the slide 1, to form a volume capable of holding fluid dispensed onto the slide 1. In this way fluid reservoir 17 enables fluid dispensed onto

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slide 1 to be held until required, without spilling off an edge of the slide. The projection 13 further assists in spreading the fluid across the full width of the cavity 18.

5 The overlap of the cavity 18 with the slide 1 forms what may be described as a reaction chamber, as illustrated in Figure 6. The cavity may vary according to application, typically from 20-200 microns. The wall portion 20 is adapted to support the cover on the slide 1. The cavityed face 22, wall portion 20 and sample holding region 5 of a slide 1 form a reaction chamber 24 when the cover 10 is placed at least partially over the sample holding region 5.

10 The fluid reservoir 17 is typically sized to be larger than the volume of the reaction chamber 24, for example 150% of the volume of the reaction chamber. This provides sufficient volume of fluid to fill the reaction chamber completely, while allowing some excess to flush the chamber, and an amount to be retained in the fluid reservoir to provide a reservoir for evaporation.

15 Clamping forces may also be applied to the cover once loaded onto the slide, and these forces are designed to provide a seal between the wall portions 20 and the upper surface of the slide 1. This is to restrict fluid leakage from the side of the cover. In one example (not shown) the wall portions may have an additional member to assist sealing of the wall portions with the upper surface 2 of the slide 1. This additional
20 member may be a softer polymer or rubber material.

The cover 10 also includes engaging surfaces in the form of wings 26. The wings 26 are adapted to engage ramps 28 of a tray 21 shown in Figure 7, to thereby lift the cover clear of the surface of the slide 1. An example of the wings lifting the cover free is shown more clearly in Figure 18. The cover 10 may be controlled by an arm
25 (not shown) moving the locating means 16. The cover 10 may be placed in a number of positions over the slide, exemplified by the positions of the cover relative to the slide shown in Figure 19. In Figure 19(a), the cover 10 is in an open position relative to the slide 1, as the sample is exposed and open. Figure 19 (b) shows the cover in a

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partially closed position, and Figure 19 (c) shows the cover in a fully closed position, where the sample is completely covered by the cover and is therefore wholly contained within the reaction chamber 24. The reaction chamber formed by the cover and cavity 18, as shown in Figure 5, extends over most of the slide 1. However it is possible that the sample may be placed more towards the end of the slide distal from the bar code 4, and therefore a smaller reaction chamber 24 is required. Reducing the size of the reaction chamber 24 reduces the amount of fluid required to fill the chamber, which can be important where expensive or scarce fluids are used. It is possible to form a smaller reaction chamber with the cover 10, by only covering a portion of the slide 1 with the cover 10. This position is shown in Figure 18 (b).

Variations in cover constructions are schematically shown in Figures 8-17. In Figures 8-17, only the front segments of the covers are shown, and the locating means 16 have been omitted from view for clarity and like parts are denoted by like reference numerals.

In Figure 8(a) a cover 10 is shown having a body 12, projecting legs 21, a protruding section 13 and an indent 30. The projecting legs 21 either side of the body 12 form a fluid receiving zone 14. When placed onto a slide, fluid may be dispensed into the fluid receiving zone, where it spreads in a circular fashion to contact the protruding section 13. The indent 30 allows the fluid to contact a wider portion of the protruding section 13 than if the front edge of the protruding section was straight (as shown in Figure 9). Once the fluid is in contact with the protruding section 13, it wicks across the width of the cavity 18. If suction is applied at the rear of the cavity, or the cover is moved along the slide from an open position to a more closed position, then the fluid begins to fill the cavity 18. When the cavity 18 has moved across the sample 3, it forms the reaction chamber 24 as the fluid may react with the sample 3.

Figures 9 (a) and (b) show a more simple construction of a cover 10 that may be used in some circumstances. The operation of the cover 10 is the same as the operation of the cover 10 in Figures 8 (a) and (b).

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Figure 10s (a) and (b) show a cover 10 having a body 12 with projecting legs 21. A protruding section 13 and a bar 31 surround a fluid receiving zone 14 for receiving fluid. The fluid may be dispensed onto the protruding section 13, where it flows down and onto the slide surface 2. The protrusion 13 and bar 31 cause the fluid to spread across the width of the cavity 18, enabling the cavity to be filled with fluid.

The covers 10 of Figures 11, 12 and 16 operate in similar ways to those described above.

In relation to all of the above-described covers, it should be appreciated that the covers are generally 25 mm across, and the cavity 18 is typically only 20-200 micrometres high. As such, overall fluid dispense volumes may be in the order of 20-300 microlitres.

Figure 13 (a) shows another cover 10 having a body 12, legs 21 and a fluid dispenser 100 dispensing fluid 102 onto the slide 1. In Figure 13 (a), the fluid 102 has already been dispensed, and has formed a fluid reservoir in the fluid reservoir 17. The schematic Figure shows a typical wicking pattern formed by the fluid as it contacts the cover 1. In Figure 13 (b), the fluid is just being dispensed onto the projection 13. In the volumes dispensed, the fluid forms a pool of comparable size to some of the cover features. Not only does the fluid flow forward of the cover as shown in Figure 13 (a), but it also flows under the cover to at least partially fill cavity 18. As mentioned above the fluid may be drawn into the cavity further by movement of the cover over the slide or suction applied to the rear of the cavity 18.

Figure 14 (a) and (b) shows a further embodiment of a cover 10 where like reference numerals are again used to denote like parts. The cover has a fluid reservoir 17, a projection 13, and a protrusion in the form of nib 15. Fluid may be deposited directly on the nib 15 so that the fluid rolls over the projection 13 into reservoir 14, and to the cavity 18, as required. If fluid is placed too far ahead of the cover, there are circumstances that may cause the fluid to reach the edge of the slide before wicking across the width of the cavity 18. It has been found that using the projection 13 causes

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the dispensed fluid to contact the covertile and spread along the full width of the cavity 13, due to the positive attraction of the covertile and the fluid. The capillary forces in the cavity cause the fluid to spread out, and the reservoir holds sufficient fluid to ensure that fluid dispensed onto the slide at least fills the cavity 18. The nib 5 15 is useful in that if the pipette is not placed to dispense the fluid accurately onto the slide, and for example misses a few millimetres in front of the projection, the nib 15 will be likely to contact the fluid, which will be drawn to the protrusion and into the reservoir. This assists in reducing bubble or void formation within the cavity. The nib 15 may extend approximately 1-5 mm from the projection 13.

10 Figure 17 shows an example of how fluid spreads across a slide when deposited in front of a cover 50. A variety of profiles for the underside of a projection 15 may be employed.

In use, a cover 10 is placed on a slide 1, as shown in Figures 4, 5 and 6 to cover the sample 3. The slide 1 will typically be in a tray 21 as shown in Figure 7, said tray 21 15 able to hold, for example, 10 slides and covers of the examples shown. The tray 21 may then be placed into a biological reaction apparatus, such as that disclosed in Australian Provisional Patent Application No. PS3114/02 by the same applicant, titled "Method and Apparatus for Providing a Reaction Chamber", filed 20 June 2002, and its associated international patent application, filed 20 June 2003, the contents of 20 which are hereby incorporated by reference.

Once the tray 21 is loaded into the apparatus (not shown) the slides 1 are held in position, typically at an angle of 5 degrees to the horizontal as shown schematically in Figures 13 (b), 15 or 17. The cover 10 is then moved by an arm (not shown) engaging the locating means 16. Typically, during a sequence referred to as an "open fill", the 25 cover 10 is moved longitudinally along the surface of the slide 1 until the sample 3 is exposed. A fluid is then dispensed by a dispensing means 100 such as a probe attached to a pump, onto the fluid receiving zone 13 (as shown in Figure 13 (b)). The amount of fluid dispensed is typically sufficient to fill the reaction chamber 24. The use of the cover 10 with this fill mechanism or methodology allows a small volume of

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fluid to be uniformly distributed across the reaction chamber 24. Distributing the fluid across the reaction chamber 24 evenly and without bubbles or air spaces allows reactions to take place on the sample 3 with greater consistency. Also, dispensing fluid into an empty receiving zone where the reaction chamber already contains fluid causes the fluid within the chamber to be replaced by the fluid in the receiving zone minimising mixing of the fluid in the reaction chamber and newly dispensed fluid. The dimensions of the reaction provide a smooth flow of fluid from the reaction chamber such that there is little mixing of the fluids. This is advantageous as it allows a previous fluid to be replaced accurately, with minimal original fluid remaining to contaminate later fluids or reactions. This reduces the number of washes required to clear the reaction chamber 24.

The volume of fluid in a reaction chamber 24 may be, for example 150 microlitres or less, although volumes may vary depending on the application and the reaction chamber dimensions.

- 15 The reaction chamber 24 is able to retain fluid due to the surface tension of the fluid, unless additional fluid is added to the fluid receiving zone, or suction is applied (typically through reduced air pressure) at the end of the slide opposite the fluid receiving zone. The reaction chamber may be filled as it is formed by the cover 10 being moved along the surface of the slide 1 to cover the sample holding region 52.
- 20 Alternatively, the reaction chamber may be filled without the cover being moved relative to the slide, due to the process of capillary wicking of dispensed fluid into the reaction chamber.

In the present examples the cover may be clamped to the slide when not in motion or retracted for an initial fill. The clamping mechanism (not shown) places force around the edge of, for example, cover 10 adjacent the wall portions 20 to locate the cover 10 with respect to the slide 1 during a reaction.

During the withdrawal of the cover 10 from the slide 1 it is sometimes desirable to remove the cover from contact with the slide. In order to accomplish this, wings 26

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engage the ramps 28 to lift the cover clear of the slide. This causes the cover 10 to lift off the slide 1 to prevent fluid contact between the slide 1 and cover 10. In this way the slide can be cleared of virtually all fluid.

5 Parts of the cover may have different material properties compared to the properties of the material of the cover body 12, which is typically plastic. In one example (not shown) the cavity may have different material properties, in order to provide a reaction chamber 24 with certain material properties. A clear plastic material has been found to be suitable for the body 12 of the cover 10, to provide suitable mechanical properties such as reasonable strength and rigidity. The cover needs to be sufficiently strong to be moved while clamping forces are applied to the cover, as the clamping forces assist in providing a sealing surface between the walls 20 of the cover 10 and the upper surface of the slide 1. The cover may be moved to empty or fill the chamber, or also, to promote fluid movement within the reaction chamber to assist a reaction.

15 The cover should ideally have some flexibility, as it is desirable that upon application of the clamp, the caved face should deflect somewhat. This has been found to assist in moving the fluid within the reaction chamber and therefore increases the exposure of the sample to the fluid.

20 Other properties of the cover 10 include the ability to restrict the heat loss from the surface of the slide 1. Typically the slide will be mounted on a heated block, and the cover will be placed over the sample on the slide. Heating the slide heats the sample and the fluid in the reaction chamber. If there is excessive heat loss from the cover 10 it is difficult to regulate the temperature of the fluid by heating the slide 1. Further, there may be an excessive temperature gradient across the reaction chamber 24, which is undesirable.

25 The caved face 19, as shown in Figure 2, may have different surface properties to the rest of the cover. It has been found to be desirable to have similar material properties for the upper surface of the slide 2 and the cavity 18. In one example, it is

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possible to coat the surface of the cavity with a material, such as silicon dioxide. This coating may be approximately 110 nm thick. The coating provides a surface with material properties similar to that of a glass slide. It has also been found that there are benefits in applying a thin layer (for example 0.5-6nm) of Chromium Oxide (Cr_2O_3) to the cavity before applying the silicon dioxide layer. This application of an intermediate layer between the silicon dioxide and plastic provides better adhesion and better thermal expansion properties for the cavity. Further, coatings in general may be used to improve the flatness of the cavity (which reduce nucleation sites and therefore bubble formation at high temperatures). The coatings may be used to modify the capillary flow characteristics of the fluid within the reaction chamber, create an impermeable barrier for gas or liquid between the cover and fluid in the reaction chamber, or provide a chemically inert surface.

In another example, it is possible to replace the caviyed face 19 with a glass insert supported by the plastic body 12 of the cover 10. It may also be possible to change the surface properties of the plastic by plasma discharge.

The covers shown in the examples may be used at temperatures approaching 100 degrees Celsius, especially when used for in-situ hybridisation reactions. At higher temperatures, the fluid evaporates and bubbles are produced. The heating may also cause the cover to bow – the cavity surface is hotter than the top of the cover and expands more, causing the cavity surface to ‘sag’ towards the slide. This helps to remove the bubbles, as the fluid wants to occupy the smaller spaces more than the bubbles do. The bubbles congregate at the ends of the cavity, and must be allowed to escape.

Experiments have demonstrated that a chamfer at the end of the cavity reliably allows the bubbles to escape to atmosphere. The existing reservoir 17 can be redesigned as illustrated in Figure 20, where a modified cover 60, similar to that shown in Figure 14, is shown with a chamfer 61 to assist in releasing bubbles, without affecting the even fluid flow through the cavity 18.. The chamfer forms a first angled section 62 at about 60° relative to the cavity and slide surface.

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Fluid evaporation rate is, however, directly linked to the surface area of the fluid exposed to atmosphere – a larger surface area will evaporate faster, and require more frequent replenishment. If the bubble escape angle is steep, the evaporation rate will increase.

- 5 This problem can be overcome by using two angles – a shallow angled section at, say, 15° between the cavity and the chamfer, to minimise evaporation, leading into the steeper angle for bubble release, which also serves to increase the volume of the reservoir.

- 10 The cover 60 is also provided with a second, identically shaped reservoir 63 at an opposite end thereof. The second reservoir 63 can also be used to replenish fluid within the cavity during heating and to allow bubbles to escape. The second reservoir 63 thereby allows for increased control of fluid conditions within the reaction chamber.

- 15 The embodiments of Figures 14 and 20 are considered to represent what is currently believed to be the best known method of performing the cover aspect of the invention.

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